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Research review paper

An overview of biological production of L-theanine

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ABSTRACT

L-Theanine (γ-glutamylethylamide) is a unique non-protein amino acid that is naturally found in tea plants. It contributes to the umami taste and unique flavor to green tea infusion, and thus its content in tea leaves highly impacts the tea quality and price. In addition to the graceful taste, it has been proved to have many beneficial physiological effects, especially promoting relaxation and improving concentration and learning ability. Based on these promising advantages, L-theanine has been commercially developed as a valuable ingredient for use in food and beverages to improve and/or maintain human health. L-Theanine can be obtained by chemical synthesis or isolation from tea, while chemical synthesis of L-theanine is hard to be accepted by consumers and is not allowed to use in food industry, and isolation of L-theanine in high purity generally involves time-consuming, cost-ineffective, and complicated operational processes. Accordingly, the biological production of L-theanine has recently attracted much attention. Four kinds of bacterial enzymes, including L-glutamine synthetase, γ-glutamylmethylamide synthetase, γ-glutamyltranspeptidase, and L-glutaminase, have been characterized to have L-theanine-producing ability. Herein, an overview of recent studies on the biological production of L-theanine was presented.

Contents

1. Introduction

It is well known that drinking tea is beneficial to health, because tea contains numerous naturally beneficial compounds, including polyphenols (Bansal et al., 2012), alkaloids (Derosa and Maffioli, 2014), catechins (Rains et al., 2011), L-theanine (Juneja et al., 1999; Vuong et al., 2011), γ-aminobutyric acid (Lin et al., 2007), vitamins, and mineral elements. L-Theanine is the most abundant free amino acid in tea, accounting for more than 50% of total free amino acid. It is the main component responsible for the flavor and taste of green tea, and thus determines the quality of green tea. In addition, a tremendous amount of research results have indicated that L-theanine may offer various favorable physiological and pharmacological effects.

L-Theanine has been approved as generally regarded as safe (GRAS) ingredient by the Food and Drug Administration (FDA). Due to the

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taste-enhancing property and promising health benefits, L-theanine is widely used in food and pharmaceutical industry. Although L-theanine can be produced by chemical synthesis or isolation from tea, biological synthesis has recently sparked a great interest as compared to the available chemical and isolation methods. The present article gives an overview of recent studies on the biological production of L-theanine.

2. Brief introduction of L-theanine

2.1. Chemical structure

Theanine (γ-glutamylethylamide or 5-N-ethyl-glutamine) is named 2-amino-4-(ethylcarbamoyl) butyric acid by International Union of Pure and Applied Chemistry (IUPAC). The molecular formula and molecular mass of theanine are $C_7H_{14}N_2O_3$ and 174.2 g mol^{-1}, respectively. Similar to other natural α-amino acid, theanine has an asymmetric carbon and thus theoretically has two chiral isomers, D- and L-theanine (Fig. 1). Theanine is biosynthesized from L-glutamic acid in plants, and therefore occurs as the L-enantiomer form in nature.

2.2. Existing sources

L-Theanine exists in a range of *Camellia* genus organisms, especially the tea-producing plants *Camellia sinensis* (Juneja et al., 1999; Vuong et al., 2011). Besides, it also has been found in mushroom *Xerocomus badius* (Casimir et al., 1960). In tea plants, L-theanine is synthesized by L-theanine synthetase (L-glutamate: ethylamine ligase, EC 6.3.1.6) from L-glutamic acid and ethylamine, and the ethylamine is produced from L-alanine by L-alanine decarboxylase (Fig. 2) (Takeo, 1974, 1978).

2.3. Health benefits

L-Theanine has been proved to have many beneficial effects to human health, including promoting relaxation (Lu et al., 2004), improving concentration and learning ability (Haskell et al., 2008), enhancing anti-tumor activity (Liu et al., 2009), preventing the vascular diseases (Rogers et al., 2008), reducing blood pressure (Yokogoshi et al., 1995), inhibiting the negative effects of caffeine (Kakuda et al., 2000), displaying neuroprotections (Egashira et al., 2007), providing anti-obesity effect (Zheng et al., 2004), improving the immune system (Miyagawa et al., 2008), and suppressing the body weight increases and fat accumulation (Takagi et al., 2010; Zheng et al., 2004).

3. Biotechnological production of L-theanine

Theoretically, L-theanine can be naturally produced by extraction and isolation from tea leaves (Lachova et al., 2007; Zhang et al., 2004). But L-theanine only composes 1–2% (*w*/*w*) of the weight of dry tea leaves (Graham, 1992). Due to lack of a simple, convenient, specific, and highly efficient isolation method, isolation of L-theanine in high purity generally involves time-consuming, cost-ineffective, and complicated operational processes.

L-Theanine can be synthesized chemically or biologically. Chemical synthesis of theanine has been widely studied, but the synthetic theanine is a racemic mixture of L- and D-forms (Gu et al., 2004; Kawagishi and Sugiyama, 1992; Lichtenstein, 1942; Yan et al., 2003).

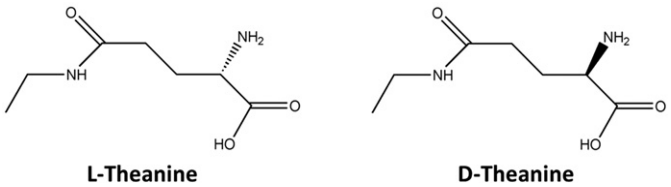

Fig. 1. Chemical structures of L- and D-theanine.

Although the chemical synthesis can offer a simple and cost-effective approach to produce theanine, with very high yield (90–700 g kg^{-1}) by various synthetic methods, the synthetic theanine is generally considered as so-called "non-natural" compound, and thus is hard to be accepted by the majority of the consumers and to be approved by national legislation.

Recently, biological production of L-theanine has attracted much attention. In tea trees, L-theanine synthetase is responsible for the L-theanine biosynthesis from glutamic acid and ethylamine (Deng et al., 2008; Sasaoka et al., 1965). The L-theanine biosynthesis was reported using the pea seed acetone powder extract containing crude L-theanine synthetase enzyme (Sasaoka et al., 1964). However, the plant L-theanine synthetase is ATP-dependent, very labile, and is hard to be isolated. For industrially biological production of L-theanine, four kinds of bacterial enzymes display potential as ideal biocatalysts, including L-glutamine synthetase (GS), γ-glutamylmethylamide synthetase (GMAS), γ-glutamyltranspeptidase (GGT), and L-glutaminase (Fig. 3).

3.1. Production of L-theanine by GS

GS (L-glutamine synthetase; L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2) mainly catalyzes the conversion of L-glutamate and free ammonium to L-glutamine with concomitant hydrolysis of adenosine triphosphate (ATP). GS is universally distributed in three domains of life, and is responsible for nitrogen metabolism and synthesis of L-glutamine, and thus for the production of amino acids, sugars, and glucosamine-6-phosphate (McCarty, 1995). In mammals, GS plays an important role in the metabolic regulation of the neurotransmitter L-glutamate (Suarez et al., 2002).

Bacterial GS can be used as biocatalyst for L-glutamine production from L-glutamate, ammonium chloride, and ATP (Tachiki et al., 1983; Wakisaka et al., 1998). Tachiki et al. (1986) first reported that GS from *Micrococcus glutamicus* ATCC 13032 could also produce L-theanine and γ-glutamylmethylamide (γ-GMA) when the substrate ammonia was substituted with ethylamine and methylamine, respectively. Then, the GS from *Pseudomonas taetrolens* Y-30 (Yamamoto et al., 2004) was also purified and characterized to have the L-theanine-producing ability. Under the optimum reaction conditions, *P. taetrolens* GS displayed 7% of the relative ligation activity toward substrate ethylamine forming L-theanine, compared to that toward ammonia forming L-glutamine (Yamamoto et al., 2004). The genes encoding the L-theanine-forming GS from *P. taetrolens* Y-30 (Yamamoto et al., 2006) were sequenced, characterized and submitted to the GenBank nucleotide sequence databases under the accession no. AB233456. The gene was cloned and expressed successfully in *Escherichia coli*. The recombinant GS displayed the same properties as those of the intrinsic GS, but the enzyme productivity in the expression system was 30-fold higher than that in original bacteria, *P. taetrolens* Y-30 (Yamamoto et al., 2006).

The ligation reaction catalyzed by bacterial GS requires a continuous supply of ATP. The regeneration of ATP is essential for the industrial applications of GS. An efficient approach to regenerate ATP was developed during the enzymatic application of GS for L-glutamine biosynthesis, which was coupled with the sugar fermentation reaction of dried baker's yeast cells. The researchers called it "coupled fermentation with energy transfer", in which glucose was used as energy source for the ATP-regenerating system during the yeast fermentation (Tachiki et al., 1983; Wakisaka et al., 1998). The coupled fermentation could also be used for L-theanine biosynthesis by GS (Fig. 4). After optimization, 100 U ml^{-1} of GS from *P. taetrolens* Y-30 could produce 170 mM of L-theanine with a yield of 28% based on the glucose consumed (Yamamoto et al., 2005).

3.2. Production of L-theanine by GMAS

Theoretically, increasing the amounts of GS in the reaction mixture could produce higher concentrations of L-theanine when the